Research Article
Preparation and Evaluation of Functional Foods for Prevention of Non-alcoholic Fatty Liver Disease

Doha A. Mohamed, Sherein S. Abdelgayed, Hend A. Essa and Rasha S. Mohamed

Department of Nutrition and Food Sciences, National Research Centre, Dokki, Cairo, Egypt
Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Egypt

Abstract
Background and Objective: Non-alcoholic fatty liver disease (NAFLD) is a public health problem presenting one of the most important common forms of liver diseases worldwide. This study was carried out to investigate the protective effect of two functional foods in form of bread containing purslane seeds meal and garden cress seeds against NAFLD. Materials and Methods: High fat and high cholesterol diet were used for induction of NAFLD in rats for 6 weeks. Plasma lipid profile (total cholesterol, triglycerides, high density lipoprotein-cholesterol and low-density lipoprotein-cholesterol, hepatic lipid profile (total fat, cholesterol, triglycerides), malondialdehyde (MDA), as well as liver (AST, ALT, total and direct bilirubin) and kidney (creatinine and urea) functions were assessed. Histological examination of liver tissue was carried out. Results: Results revealed that significant elevation in plasma and liver lipid profiles, MDA, liver enzymes (AST and ALT), bilirubin (total and direct) and kidney function (creatinine and urea) were observed in NAFLD control compared to normal control. Feeding rats on diet containing functional food I and II (purslane and garden cress bread, respectively) showed significant improvement in all the studied parameters with remarkable effect regards to functional food I (purslane bread). Conclusion: Purslane bread and garden cress bread as functional foods prepared in the present study prevent weight gain, improve plasma lipid profile and prevent hepatic lipid accumulation effectively in NAFLD model in rats. Also decreased lipid peroxidation, improve liver and kidney functions and possess hypoglycemic effect. Purslane bread was superior in the prevention of hepatic lipid accumulation.

Key words: Functional foods, non-alcoholic fatty liver, purslane bread, garden cress bread, rats, high fat diet

Citation: Doha A. Mohamed, Sherein S. Abdelgayed, Hend A. Essa and Rasha S. Mohamed, 2018. Preparation and evaluation of functional foods for prevention of non-alcoholic fatty liver disease. Pak. J. Biol. Sci., CC: CC-CC.

Corresponding Author: Doha A. Mohamed, Department of Nutrition and Food Sciences, National Research Centre, Cairo, Egypt

Copyright: © 2018 Doha A. Mohamed et al. This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.
INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a systemic upset of energy, glucose and lipid homeostasis with hepatic appearance\(^1\). The NAFLD is increasingly predominant and substantially correlating with obesity, diabetes and the metabolic syndrome all over the world in the 21st century\(^2\). The NAFLD can be defined by accumulation of fat in liver in the absence of excessive alcohol consumption and any other specific causes of hepatic steatosis like viral hepatitis, autoimmune hepatitis and others\(^3,4\). The NAFLD is rapidly becoming the most common cause of chronic liver disease with an estimated worldwide prevalence of 25.2% due to an increase in the prevalence of obesity\(^5\). The NAFLD may be progress to steatohepatitis, fibrosis, cirrhosis and liver cancer as a result of excessive and continues accumulation of fat in liver that lead to elevate oxidative stress and inflammation\(^6\). The prevalence of hyperlipidemia/dyslipidemia in NAFLD patients\(^7\) is 69.5%, so the most common cause of death in patients with NAFLD is cardiovascular disease\(^8\). Lifestyle modification is the cornerstone of treatment intervention for patients with NAFLD, which includes diet modification, sustained weight loss 5-10% and increase in physical activity/exercise\(^9\). Modification in the diet is one of the ways to prevent or cure NAFLD. Diet containing functional foods ingredients such as polyunsaturated fatty acids, dietary fibers and phenolic compounds is a meaningful concept to treat or protect from NAFLD. The management of NAFLD should be associated with cure or prevent its metabolic comorbidities. So any strategy for treatment or prevent NAFLD must take in consideration all the metabolic syndrome diseases such as diabetes and dyslipidemia. Plant foods, seeds and food rich in anti-oxidant, anti-inflammatory and having lipid lowering effect may have an efficient effect in protection and improvement of NAFLD\(^10,10\). Purslane, called ‘Rejlah, in Arabic (Portulaca oleracea L.) is one of the most popular herbs which is a good source of biologically active compounds such as omega-3, α-tocopherol, ascorbic acid, β-carotene\(^11,12\), glutathione, minerals, phenolic and flavonoids compounds\(^13\). Garden cress seeds (Lepidium sativum Linn.) family Brassicaceae are rich source of phytochemicals including phenolic compounds, alkaloids, flavonoids; they are also highly nutritive (contain 22.5% protein, 27.5% fat, 30% dietary fiber) and contain many vitamins and minerals like ascorbic acid, tocopherol, calcium, iron and polyunsaturated fatty acids such as linoleic (7.6%) and linolenic (29.3%)\(^14,15\). Purslane and garden cress are traditional and popular herbs and still frequently consumed in Egypt. For treatment of NAFLD modification in dietary habites, exercise and type of food plays an important role. Few literatures are available for preparation of functional food for treatment or prevention NAFLD. So the present research is a new idea for treatment or prevention of NFLD and there is no previous studies for preparation of functional foods contained the same ingredients used in the prepared functional foods in the present study. This study aimed preparation and evaluation of two functional foods in form of bakery product (bread) containing purslane seeds meal and garden cress seeds against NAFLD.

MATERIALS AND METHODS

Materials

Plant materials: Garden cress seeds, purslane seeds, wheat germ, oat, sunflower seeds, skim milk, yeast and whole wheat flour were purchased from local markets, Cairo, Egypt.

Animals: Male Sprague Dawley rats weighing 100-128 g (113.8±8.381 as Mean±SD) were used in the present study. Animals were obtained from Animal house of National Research Centre, Cairo, Egypt. Animals were kept individually in stainless steel metabolic cages, water and food were given ad libitum.

Proximate composition of the prepared functional foods: Moisture, protein, fat, crude fiber and ash of the prepared functional foods were determined according to AOAC\(^16\). Carbohydrates were calculated by differences.

Diets: Experimental diets were prepared as in Table 1. High fat and high cholesterol diet were used for induction of fatty liver disease in rats according to the method of Zhu et al.\(^17\) with modification by using coconut oil as source of fat instead of lard. Salt mixture and vitamin mixtures were prepared according to Briggs and Williams\(^18\) and Morcos\(^19\), respectively. Oil soluble vitamins were given orally in a dose of 0.1 mL/rat per week. Functional foods were dried and grind into powder before being added to the diets.

Methods

Preparation of purslane seeds meal: Purslane seeds were crushed and pressed with laboratory type of (screw press with speed 15 rpm and 35 c) carver hydraulic press under 10,000 lb/in (pic) pressure for 1 h at room temperature according to the method of Ustun et al.\(^20\). The produced meal was kept in deep-freeze until used.
absolute liver weight (g) Relative liver weight = 100
Final body weight (g)

Preparation of functional foods: Two functional foods (I and II) were prepared in form of bread for prevention of NAFLD. Ingredients of bread I were purslane seeds meal, oat, wheat germ, skim milk, yeast and whole wheat flour. Ingredients of bread II were garden cress seeds, skim milk, flax seeds, oat, sunflower seeds, yeast and whole wheat flour.

Design of the animal experiment: Twenty four rats were divided into four groups each comprised 6 rats. The first was normal group where rats were received a balanced diet all over the study period for 6 weeks, while group two was the NAFLD group, where rats were fed on high fat high cholesterol diet all over the study period, this group served as NAFLD control. Rats in group three and four were fed on high fat high cholesterol diet containing 20% purslane bread or garden cress bread all over the study period. During the experiment, body weight and food intake were recorded once weekly. At the end of the study total food intake, body weight gain and feed efficiency ratio (Body weight gain/total food intake) were calculated. This study has been carried out according to the Medical Research Ethics Committee, National Research Centre, Cairo, Egypt and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Oral glucose tolerance test-OGTT: The glucose tolerance of the rats was assessed by the oral glucose tolerance test (OGTT). This test was performed during the last week of the experiment after 12 h of fasting and began when the first blood sample was taken from a cut at the tip of the tail (time 0). Subsequently, a 20% glucose solution (2 g kg-1 rat b.w.t.) was administered to the rats via stomach tube. Blood samples were collected for determination of glucose level after 30, 60 and 120 min post-glucose treatment using Accu-Chek Active (Accu-Chek, Mannheim, Germany) in a drop of blood from the tail22.

Blood and tissue sampling: Blood samples were collected from all rats after an overnight fasting at the end of the experiment (6 weeks) for the determination of total cholesterol (T-Ch)23, high density lipoprotein-cholesterol (HDL-Ch)23, low-density lipoprotein-cholesterol (LDL-Ch)24 and triglycerides (TG)25. The T-Ch/HDL-Ch ratio was calculated. Plasma malondialdehyde (MDA) was determined as indicator of lipid peroxidation26. The activity of aspartate transaminase (AST)27, alanine transaminase (ALT)27 and plasma total and direct bilirubin28 were determined as indicator of liver function. Plasma level of creatinine29 and urea30 were determined as indicator of kidney function. Liver was immediately removed, weighed and stored at -20°C till analyzed. For histopathological study, part of liver was removed, placed in 10% formaldehyde, dehydrated in graded alcohol and embedded in paraffin. Fine sections were prepared, mounted on glass slides and counter-stained with hematoxylin and eosin for light microscopic analysis31. Relative weight of liver of each animal was calculated as follows:

Relative liver weight = \frac{\text{Absolute liver weight (g)}}{\text{Final body weight (g)}} \times 100

Extraction and determination of hepatic lipids: Total hepatic lipids were extracted and weighed according to the procedure of Folch et al.25. In brief, liver tissues were homogenized with chloroform:methanol (2:1) for lipids extraction. After the extraction and evaporation, tissue lipids were re-dissolved in isopropanol and liver cholesterol22 and triglyceride25 levels were estimated enzymatically.

Statistical analysis: The results of animal experiments were expressed as the Mean±SE and they were analyzed statistically using the one-way analysis of variance ANOVA followed by Duncan’s test. In all cases, p<0.05 was used as the criterion of statistical significance.
RESULTS

Proximate composition of functional foods: Proximate compositions of the bread samples (Table 2) revealed that garden cress bread was significantly higher in all the determined parameters, protein, fat and ash, than purslane bread except for carbohydrate content, which was significantly higher in the purslane bread (69.1%) than garden cress bread (62.2%). Protein was present in garden cress bread and purslane bread by 30.9 and 26.7%, respectively. Percentage of ash in garden cress bread was 2.5%, while in purslane bread was 2%. Fat content in garden cress bread was 3.1%, while in purslane bread was 1.2%. Fibers content of garden cress bread (1.3%) were significantly higher than purslane bread (1%).

Oral glucose tolerance test (OGTT): After 6 weeks of the study OGGT were carried out. The results of OGGTTs (Fig. 1) revealed that NAFLD control showed the highest elevation of glucose after 1 h of glucose administration compared to the different experimental groups. Normal rats group and rats groups fed on high fat high cholesterol diet containing functional foods (purslane or garden cress bread) showed the highest elevation in glucose levels after 30 min from glucose administration.

Nutritional and biochemical parameters of the different experimental groups: Table 3 presented biochemical parameters of different experimental groups. The NAFLD rats clarified significant dyslipidemia declared as an elevation in plasma triglycerides, total cholesterol, LDL-Ch, T-Ch/HDL-Ch ratio and reduction in HDL-Ch when compared to normal control group. The NAFLD group showed significant elevation in hepatic total fat, total cholesterol and triglycerides by 55.33, 9.20, 17.16 mg g⁻¹ tissue, respectively when compared with normal control group. Both groups of rats feeding on diet containing purslane or garden cress bread showed significant improvement in plasma lipid profile and hepatic fat with different degrees compared to NAFLD group but still significantly differ from normal group. Purslane bread was more effective in reduction of hepatic fat than garden cress bread. The NAFLD group showed significant elevation in liver function as represented by significant elevation of bilirubin (total and direct) and the activities of transaminases (ALT and AST). Administration of purslane or garden cress bread in the diet significantly reduced bilirubin and the activities of transaminases. Plasma MDA as indicator of lipid peroxidation elevated significantly in NAFLD group compared with normal group. Rats feeding on diet containing purslane or garden cress bread as functional foods reduced MDA plasma levels significantly. Creatinine and urea as indicator to kidney function elevated significantly in NAFLD group compared with normal group. Feeding rats on diet containing purslane or garden cress bread reduced the elevation of kidney function to reach normal values.

Nutritional parameters of the different experimental groups were presented in Table 4. The results revealed that high fat high cholesterol diet produced significant elevation in body weight gain and in relative liver weight in NAFLD control compared to normal control group. Feeding on diet containing functional food I or II (purslane bread and garden cress bread, respectively) exhibited reduction in body weight gain with significant improvement regard to garden cress bread.

Histopathological examination of the liver tissue: Liver of normal control group showed normal parenchyma of hepatic cords, blood sinusoids and portal areas (Fig. 2a). Liver of NAFLD group showed diffuse fatty degenerated hepatocytes, large and massively diffused circumscribed hepatic vacuoles with signet ring appearance (arrows) were noted (Fig. 2b). Liver of NAFLD rats group fed on diet containing purslane
Fig. 2(a-d): Section of rat liver of different groups (H and E X400), (a) Liver of rat of the normal control group, (b) Liver of the NAFLD group, (c) Liver of NAFLD purslane bread group and (d) Liver of NAFLD garden cress bread group

Table 3: Biochemical parameters of different experimental groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal control</th>
<th>Fatty liver control</th>
<th>Purslane bread group</th>
<th>Garden cress bread group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-Ch (mg dl⁻¹)</td>
<td>74.40±3.47ᵃ</td>
<td>102.95±2.63ᵇ</td>
<td>85.03±3.78ᵇ</td>
<td>90.69±3.21ᵇ</td>
</tr>
<tr>
<td>TG (mg dl⁻¹)</td>
<td>65.33±2.00ᵃ</td>
<td>162.33±3.92ᵇ</td>
<td>73.44±3.49ᵇ</td>
<td>81.48±1.47ᵇ</td>
</tr>
<tr>
<td>HDL-Ch (mg dl⁻¹)</td>
<td>39.00±0.96ᵃ</td>
<td>24.20±0.66ᵇ</td>
<td>33.40±1.24ᵇ</td>
<td>32.83±0.94ᵇ</td>
</tr>
<tr>
<td>LDL-Ch (mg dl⁻¹)</td>
<td>20.72±0.35ᵃ</td>
<td>55.66±2.40ᵇ</td>
<td>39.43±2.58ᵇ</td>
<td>39.83±1.40ᵇ</td>
</tr>
<tr>
<td>T-Ch/HDL-Ch ratio</td>
<td>1.91±0.10ᵃ</td>
<td>4.25±0.08ᵇ</td>
<td>2.56±0.16ᶜ</td>
<td>2.77±0.13ᶜ</td>
</tr>
<tr>
<td>MDA (mmol L⁻¹)</td>
<td>5.02±0.25ᵃ</td>
<td>7.41±0.41ᵇ</td>
<td>4.90±0.31ᵇ</td>
<td>5.11±0.21ᵇ</td>
</tr>
<tr>
<td>Total bilirubin (mg dl⁻¹)</td>
<td>2.20±0.08ᵇ</td>
<td>2.88±0.07ᵇ</td>
<td>2.39±0.08ᵇ</td>
<td>2.38±0.09ᵇ</td>
</tr>
<tr>
<td>Direct bilirubin (mg dl⁻¹)</td>
<td>1.34±0.03ᵇ</td>
<td>1.80±0.03ᵇ</td>
<td>1.41±0.04ᵇ</td>
<td>1.40±0.03ᵇ</td>
</tr>
<tr>
<td>ALT (IU L⁻¹)</td>
<td>21.66±0.91ᵃ</td>
<td>28.33±1.05ᵇ</td>
<td>25.66±0.66ᵇ</td>
<td>25.83±0.87ᵇ</td>
</tr>
<tr>
<td>AST (IU L⁻¹)</td>
<td>42.33±2.27ᵃ</td>
<td>73.50±3.52ᵇ</td>
<td>46.16±1.13ᶜ</td>
<td>48.50±1.43ᶜ</td>
</tr>
<tr>
<td>Creatinine (mg dl⁻¹)</td>
<td>0.48±0.02ᵇ</td>
<td>0.66±0.03ᵇ</td>
<td>0.53±0.03ᵇ</td>
<td>0.57±0.02ᵇ</td>
</tr>
<tr>
<td>Urea (mg dl⁻¹)</td>
<td>27.30±0.83ᵃ</td>
<td>30.89±0.93ᵇ</td>
<td>29.60±0.641ᵇ</td>
<td>28.43±1.34ᵇ</td>
</tr>
<tr>
<td><strong>Liver tissue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total fat (mg g⁻¹ tissue)</td>
<td>21.00±0.73ᵃ</td>
<td>55.33±1.25ᵇ</td>
<td>22.66±0.88ᵇ</td>
<td>24.83±0.79ᵇ</td>
</tr>
<tr>
<td>T-Ch (mg g⁻¹ tissue)</td>
<td>2.11±0.10ᵃ</td>
<td>9.20±0.20ᵇ</td>
<td>2.65±0.19ᵇ</td>
<td>3.27±0.17ᵇ</td>
</tr>
<tr>
<td>TG (mg g⁻¹ tissue)</td>
<td>5.21±0.20ᵃ</td>
<td>17.16±0.70ᵇ</td>
<td>5.90±0.27ᵇ</td>
<td>6.63±0.31ᵇ</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SE. Values with different superscript letters in the same raw are significantly different at p<0.05 levels.

Table 4: Nutritional parameters of different experimental groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal control</th>
<th>Fatty liver control</th>
<th>Purslane bread group</th>
<th>Garden cress bread group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>113.70±3.06ᵃ</td>
<td>113.50±6.91ᵇ</td>
<td>113.50±4.47ᵇ</td>
<td>113.70±3.03ᵃ</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>113.50±6.91ᵇ</td>
<td>205.60±6.46ᵃ</td>
<td>165.50±3.13ᵇ</td>
<td>157.20±3.55ᵇ</td>
</tr>
<tr>
<td>Body weight gain (g)</td>
<td>59.20±4.85ᵇ</td>
<td>92.10±6.07ᵇ</td>
<td>51.90±5.21ᵇ</td>
<td>43.50±3.79ᵇ</td>
</tr>
<tr>
<td>Total food intake (g)</td>
<td>739.80±4.91ᵃ</td>
<td>762.00±6.07ᵇ</td>
<td>665.90±5.81ᵇ</td>
<td>632.90±6.04ᵇ</td>
</tr>
<tr>
<td>Feed efficiency ratio</td>
<td>0.15±0.004ᵇ</td>
<td>0.14±0.005ᵇ</td>
<td>0.17±0.005ᵇ</td>
<td>0.17±0.006ᵇ</td>
</tr>
<tr>
<td>Relative liver weight</td>
<td>2.79±0.12ᵇ</td>
<td>3.57±0.21ᵇ</td>
<td>3.79±0.23ᵇ</td>
<td>3.73±0.24ᵇ</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SE. Values with different superscript letters in the same raw are significantly different at p<0.05 levels.

Bread showed marked improvement of hepatic parenchyma, healthy hepatocytes with normal blood sinusoids were noted (Fig. 2c). Liver of NAFLD rats group fed on diet containing garden cress bread showed moderate improvement of hepatic parenchyma and few focally scattered number of vacuolated hepatocytes (arrows) were noted (Fig. 2d).

**DISCUSSION**

In the current study high fat and high cholesterol diet was used for induction of NAFLD in rats. It was reported previously that prolonged exposure to high fat diet lead to NAFLD in rats. In the present research refined coconut oil was used as
the source of fat for induction of NAFLD. Coconut oil is rich in saturated fatty acids (92.13%), especially lauric (42.67%) and myristic (21.12%) as reported by Wall-Medrano et al.\textsuperscript{14}. It was reported previously that coconut oil induced hypercholesterolemia and hypertriglyceridemia in rats\textsuperscript{35,36} and humans\textsuperscript{37}.

In the current research feeding rats on high fat diet induced NAFLD as observed by dyslipidemia and increased hepatic lipid, which associated with elevation of plasma bilirubin (total and direct) and the activities of transaminases (AST and ALT). Also, histopathological changes observed in liver tissue in the present study proved the presence of NAFLD. The histopathological results matched with the biochemical results. These changes prove the induction of NAFLD by this diet. NAFLD was associated with elevation of MDA as indicator of lipid peroxidation. The increment in MDA levels plays an important role in liver damage\textsuperscript{38}. The present result is in accordance with the results of Zhu et al\textsuperscript{19} who reported that high fat diet increase MDA as indicator of lipid peroxidation and oxidative stress in plasma and liver tissue. Elevation the activities of transaminases (ALT, AST) in rats feeding on high fat high cholesterol diet are in agreement with the results of Al-Hamedan\textsuperscript{40} and Chauhan et al\textsuperscript{41}.

Functional foods research is an emerging era in the prevention and treatment of chronic diseases\textsuperscript{42}. Two functional foods were prepared and used in the present study. Both functional foods prepared in the current research contain phytochemicals such as phenolic compounds and phytonutrient such as omega-3 fatty acids and dietary fibers. All these phytochemicals and phytonutrient possesses beneficial effect towards treatment and protection against chronic diseases. In the present study feeding rats on high fat and high cholesterol diet containing 20% of functional food I or II (purslane bread or garden cress bread, respectively) improve all the studied parameters with different degrees. The improvement in dyslipidemia in rats feeding on both functional foods as observed by reduction of T-Ch, TG, LDL-Ch and the ratio of T-Ch/HDL-Ch in accordance with elevation of HDL-Ch and also the reduction of hepatic lipid. The improvement of dyslipidemia in the present study was associated with reduction of lipid peroxidation through reduction of MDA. The improvement in all the studied biochemical markers is associated with significant reduction in fat appearance in the liver tissue in histopathological examination and also improvement in the studied nutritional parameters. The present results are in agreement with the results of Al-Hamedan\textsuperscript{40}, El-Sayed et al\textsuperscript{43} and Sultana and Rahman\textsuperscript{44}, who reported the protective effect of purslane and garden cress seeds extract and powder on hypercholesterolemic rats. The improvement in all the indicators of NAFLD in the present study may be attributed to the presence of phenolic compound, dietary fibers and polyunsaturated fatty acids in the prepared functional foods.

In the present research, MDA was significantly decreased in rats feeding on diet containing food functional I or II. These results demonstrated that both functional foods prepared in the present study can improve the activity of anti-oxidant enzymes in the liver of NAFLD rats and reduce the content of lipid peroxidation products. So the prepared functional foods protected liver by mitigating the degree of lipid peroxidation, reducing the degree of liver cell damage and degeneration and promoting its regeneration. Purslane has the ability to inhibit lipid peroxidation by scavenging free radicals and increasing intracellular concentration of glutathione due to the presence of flavonoids, omega-3, ascorbic acid, β-carotene and glutathione\textsuperscript{45}. Garden cress seeds reduced MDA as indicator of lipid peroxidation as reported by Chauhan et al\textsuperscript{41} due to presence of phenolic compounds, alkaloids, flavonoids and their derivatives, which possess anti-oxidant potential, anti-inflammatory, anti-cancer and cardio-protective activit\textsuperscript{15,46}.

The studied functional foods reduced glucose in oral glucose tolerance compared with NAFLD rats group. This reduction in glucose level is a good result that these functional foods could be used also for type-2 diabetes as one of the metabolic syndrome diseases related to NAFLD. Dietary fiber in the prepared functional food reduced of glucose absorption which leads to decrease insulin secretion and prevent the risk of hypoglycemia during post-absorption period and reducing hunger\textsuperscript{47}.

Total food intake was reduced significantly in rats feeding diet containing purslane or garden cress bread. The observed reduction in food intake may be due to presence of dietary fibers in both functional foods, which increase satiety feeling (reducing hunger) and also reduced lipid profile through increase cholesterol excretion in the feces. Dietary fiber in the small intestine delay intestinal transit and reduce glucose and free fatty acid absorption with a consequent increment in fat oxidation and reduction in fat storage\textsuperscript{48}. Also it was reported that fiber-rich meal may favour the release of cholecystokinin, a peptide involved in gastric emptying regulation and hypothalamic satiety nucleus stimulation\textsuperscript{49}. Dietary fiber increase glucagon-like peptide-1, a hormone in the gut involved in satiety control, gastric emptying and small intestine transit\textsuperscript{50}. Dietary fiber ferment in the large intestine by intestinal bacteria and influences gut microbiota.
composition and produce short chain fatty acids which positively influence body weight regulation\textsuperscript{15,52}. Dietary fibers regulate body weight through decreasing gastric emptying and prolonging satiety, improving insulin sensitivity and modulating glucose and lipid oxidation\textsuperscript{53}. Dietary fiber beneficial impact on gut microbiota could explain the possible fiber effect on body weight regulation mediated by increasing caloric extraction from food\textsuperscript{54}.

The protective effect of the two functional foods prepared in the form of breads (purslane and garden cress bread) in the present study towards NAFLD may be attributed to presence of phenolic compound where phenolic compounds were documented to reduce NAFLD and cardiovascular disease\textsuperscript{55}. Phenolic compounds possess anti-oxidant, anti-diabetic and anti-inflammatory activities\textsuperscript{56-58}. Also polyphenol rich compounds decreased intestinal absorption of triglycerides by inhibition of pancreatic lipase\textsuperscript{59}. Flavonoids reduced TCh, LDL-Ch and VLDL-Ch and increased HDL-Ch due to increase lipolysis more than hypogeneration\textsuperscript{60}.

The studied functional foods contain polyunsaturated fatty acids (PUFAs) as functional food ingredient present in the food sources used in the preparation of the functional foods in the present research. These PUFAs especially omega-3 fatty acids play an important role as hypotriglyceridemic, anti-diabetic and anti-inflammatory\textsuperscript{61}. Long term supplementation with omega-3 fatty acids reduced levels of AST and ALT in children with no-side effects\textsuperscript{62}. The PUFAs inhibit lipogenesis and induce fatty acid oxidation in liver and adipose tissue via regulation of key transcription factors such as the peroxisome proliferator-activated receptors and sterol regulatory element binding protein\textsuperscript{63}. In the present research purslane bread and garden cress bread contain high levels of plant protein. It was reported previously that plant protein play an important role in the management of NAFLD\textsuperscript{64}.

**CONCLUSION**

Purslane bread and garden cress bread as functional foods prepared in the present study prevent weight gain, improve plasma lipid profile and prevent hepatic lipid accumulation effectively in NAFLD model in rats. Also decreased lipid peroxidation, improve liver and kidney functions and possess hypoglycemic effect. Purslane bread was superior in the prevention of hepatic lipid accumulation. The beneficial effect of the functional foods in the present research may be attributed to the presence of dietary fibers, phenolic compounds, plant protein and polyunsaturated fatty acids as functional food ingredients.

**SIGNIFICANCE STATEMENT**

This study confirmed that purslane bread and garden cress bread as functional foods are good strategy for prevention of NAFLD in rats fed on high fat diet. Both functional foods reduced weight gain, improve dyslipidemia, reduced lipid peroxidation and prevent hepatic lipid accumulation effectively. Purslane bread was superior in the prevention of hepatic lipid accumulation.

**REFERENCES**


